

FIG. 1

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OP-1	CCA - - PTQLNAI SVLYFDDS- SNVI LKKYRNMVVRA	CGCH
BMP-5	CCA - - PTKLNAI SVLYFDDS- SNVI LKKYRNMVVRS	CGCH
BMP-6	CCA - - PTKLNAI SVLYFDDN- SNVI LKKYRNMVVRA	CGCH
OP-2	CCA - - PTKLSATSVLYYDSS- NNVI LRKHRNMVVKAC	CGCH
OP-3	CCV - - PTELSAI SLLYYDRN- NNVI LRRERNMVVQAC	CGCH
60A	CCA - - PTRLGALPVL YHLND- ENVNLKKYRNMI VKS	CGCH
Vg-1	CCV - - PTKMSPI SMLFYDNN- DNVVL RHYENMAVDE	CGCR
UNIVIN	CCA - - PTKLSGI SMLYFDNN- ENVVL RQYEDMVVEA	CGCR
BMP-2	CCV - - PTELSAI SMLYLDEN- EKVV LKNYQDMVVEG	CGCR
BMP-4	CCV - - PTELSAI SMLYLDEY- DKVV LKNYQEMVVEG	CGCR
GDF-5	CCV - - PTRLSPI SILFIDSA- NNVVYKQYEDMVVES	CGCR
GDF-6	CCV - - PTKLTPI SILYIDAG- NNVVYKQYEDMVVES	CGCR
GDF-7	CCV - - PARLSPI SILYIDAA- NNVVYKQYEDMVVEA	CGCR
CDMP-2	CCV - - PTKLTPI SILYIDAG- NNVVYNEYEEMVVES	CGCR
dpp	CCV - - PTQLDSVAMLYLNDQ- STVV LKNYQEMTVVG	CGCR
BMP-9	CCV - - PTKLSPI SVLYKDDMGVPTLKYHYEGMSVAE	CGCR
DORSALIN	CCV - - PTKLDAI SILYKDDAGVPTLIYNYEGMKVAE	CGCR
BMP-10	CCV - - PTKLEPI SILYLDKG- VVTYKFKYEGMAVSE	CGCR
GDF-3	VCV - - PTKLSPI SMLYQDSD- KNVI LRHYEDMVVDE	CGCG
GDF-1	CCV - - PERLSPI SVLFFDNE- DNVVL RHYEDMVVDE	CGCR
SCREW	CCV - - PTVLGAI TILRYLNE- DIIDLTKYQKAVAKE	CGCH
BMP-3	CCV - - PEKMSSL SILFFDEN- KNVVLKVYPNMTVES	CACR
NODAL	CCA - - PVKTKPLSMLYVDN- - GRVLL EHHKDMI VEE	CGCL
TGF- α 2	CCV - - SQDLEPLTILYYIG- - KTPKI EQLSNMI VKS	CKCS
TGF- α 3	CCV - - PQDLEPLTILYYVG- - RTPKVEQLSNMVVK	CKCS
TGF- α 4	CCV - - PQTLDPLPIIYYVG- - RNV RVEQLSNMVVRAC	CKCS
TGF- α 1	CCV - - PQALEPLPIVYYVG- - RKP KVEQLSNMI VRS	CKCS
TGF- α 5	CCV - - PDVLEPLPIIYYVG- - RTAKVEQLSNMVVRS	CNCS
GDF-9	SCV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR
Inhibin α	CCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQH	CACI
Inhibin β A	CCV - - PTKLRPMSMLYYDDG- QNI I KKDI QNMI VEE	CGCS
Inhibin β B	CCI - - PTKLSTMSMLYFDDE- YNI VKRDVPNMI VEE	CGCA
Inhibin β C	CCV - - PTARRPLSLLYYDRD- SNI VKTDI PDMVVEA	CGCS
MIS	CCV - - PTATAGKLLI SLSE- - ERI SAHHVPNMVATE	CGCR
GDNF	CCR - - PIAFDDD- - LSFLD- - DNLVYHI LRKHS	AKRCGCI
BMP-11	CCT - - PTKMSPINMLYFNDK- QQI I YGKI PGMVVDRC	CGCS
GDF-9	SCV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR

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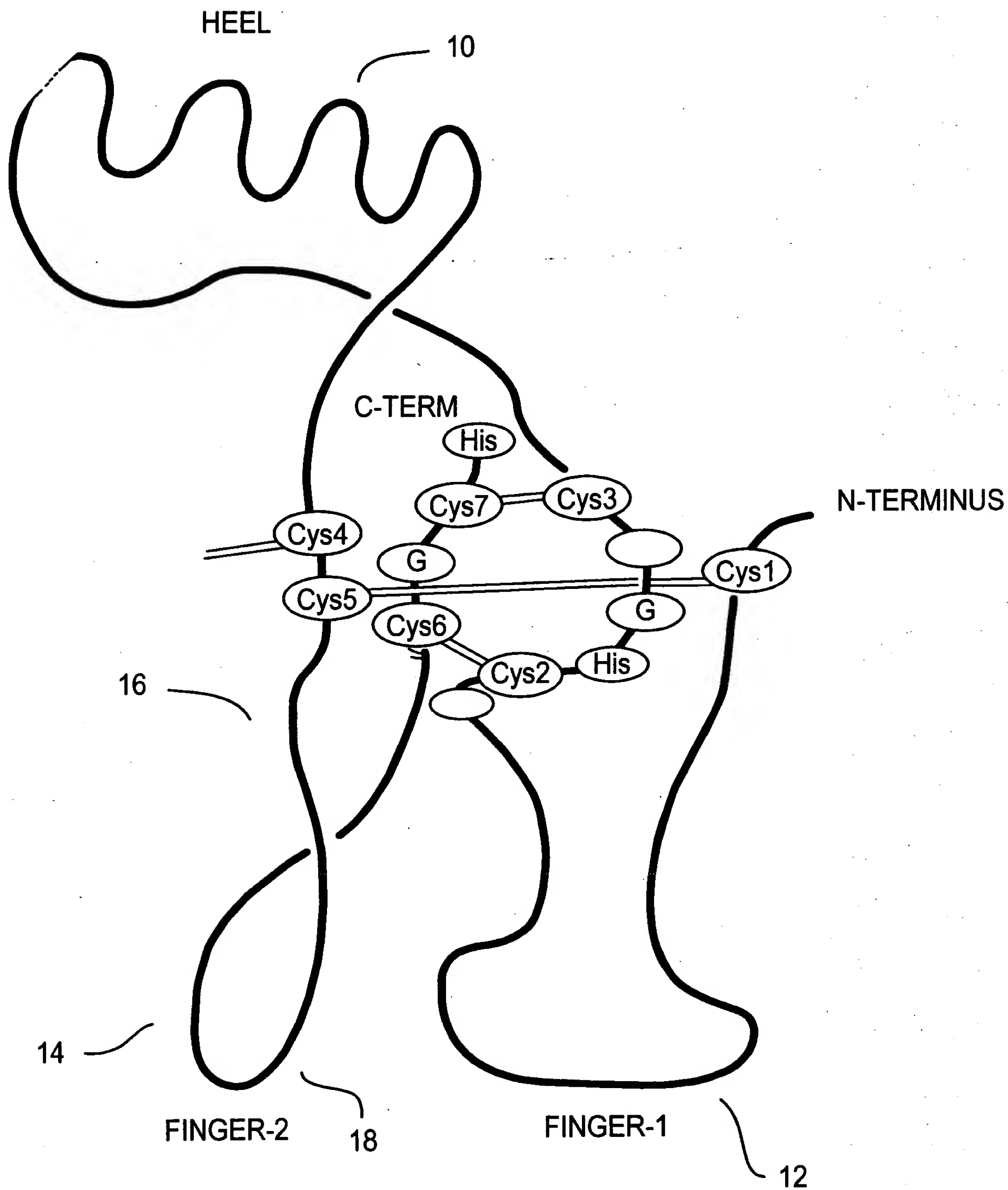


FIG.2

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7-CYSTEINE DOMAIN OF OP-1

FINGER-1

TGTAAGACGAGCTGTATGTCAGCTCCGAGACCTGGGCTGCCAGACTGGATCATCGCGCCTGAAGGCTACGCCGCCCTACTACTGTGAGGGG
C K K H E L Y V S F R D L G W Q D W I I A P E G Y A A Y Y C E G

HEEL

GAGTGTGCCCTCCCTCTGAACCTCTACATGAACGCCACCAACCCACCTCGTGCAGACGCTGGTCCACTTCATCAACCCGGAAACGGTGCCCAAGCCCTGC
E C A F P L N S Y M N A T N H A I V Q T L V H F I N P E T V P K P C

FINGER-2

TGTGCGCCACGAGCTCAATGCCATCTCCGTCCTCTACTTCGATGACAGCTCCAACGTCATCCTGAAGAAATACAGAAACATGGTGGTCCGGGCCTGTGGCTGCCAC
C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H

FIG. 3

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OP-1 CHIMERICS WITH CDMP-2 OR WITH BMP-2

PARENTAL MOLECULES:

REFOLDING ACTIVITY (CELL BASED)

	FINGER1	HEEL	FINGER2			
OP-1				h	(-)	+++ (*)
BMP-2				r	+++	+++
CDMP-2				r	++++	+/-

REPLACING FINGER-1 OR HEEL:

H2383				r	+/-	N/A
H2362				r	+	N/A
H2360				r	+	N/A
H2331				r	+	N/A

REPLACING FINGER-2 OR HEEL:

H2389				r	+++	+++
H2471				r	+++	+++
H2388				r	+++	+/-
H2410				r	+++	+++
H2429				r	+/-	N/A

CHANGING PATCHES OF RESIDUES:

H2381				r	+++	N/A
H2390				r	+	N/A
H2396				r	+	N/A
H2421				r	+/-	N/A

PAIRED CHANGES IN FINGER-2:

H2418				r	+++	++
H2420				r	++++	+/-

FIG. 4A

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OP-1 MUTANTS WITH C-TERMINAL ARGININE INSTEAD OF HISTIDINE:

H2247		+	+++
H2233		+	+++

BALANCING OF CHARGED RESIDUES IN FINGER-2 OF OP-1 MUTANTS:






H2406		+/-	N/A
H2443		+++	++
H2447		+++	++
H2433		+/-	N/A
H2456		+++	+++

FIG. 4B

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CORRELATION OF REFOLDING EFFICIENCY AND CHARGED AMINO ACIDS
IN THE TGF- β (SEVEN CYSTEINE) DOMAIN

PROTEIN	FINGER-1	CXGXC	HEEL	FINGER-2	CXCX C-TERM	TOTAL OF CHARGED RESIDUES (+), (-) = TOTAL	NEGATIVE CHARGES, FINGER-2	NET CHARGES, FINGER-2	REFOLDING EFFICIENCY
OP-1	3+, 4-	2-	1+, 1-	4+, 2-	0	8+, 9- = 17	2-	2+	+/-
H2247	3+, 4-	2-	1+, 1-	4+, 2-	1+	9+, 9- = 18	2-	2+	+
H2447	3+, 4-	2-	1+, 1-	2+, 6-	1+	7+, 12- = 19	6-	4-	+++
BMP-3	4+, 4-	0	3+, 1-	3+, 4-	1+	11+, 9- = 20	4-	1-	+++
BMP-2	2+, 3-	1-	2+, 1-	2+, 6-	1+	7+, 11- = 18	6-	4-	+++
GDF-5	3+, 5-	1-	1+, 4-	2+, 4-	1+	6+, 14- = 20	4-	2-	+++
CDMP-2	3+, 5-	1-	1+, 3-	2+, 4-	1+	6+, 13- = 19	4-	2-	+++
GDNF	2+, 4-	0	6+, 4-	5+, 5-	0	13+, 13- = 26	5-	0	+++
TGF- β 1	5+, 3-	0	1+, 1-	5+, 2-	1+	11+, 6- = 17	2-	3+	+/-
TGF- β 2	5+, 3-	0	1+, 2-	4+, 3-	1+	10+, 8- = 18	3-	1+	+/-

FIG. 5

FIG. 6A

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TGF-β SUBGROUP-----+-----+-----+																																										
TGF-β1:	P	Y	I	W	S	-	-	-	-	-	-	L	D	T	Q	Y	S	K	V	L	A	L	Y	N	Q	H	N	P	-	-	G	A	S	A	A	P		C		C		
TGF-β2:	P	Y	L	W	S	-	-	-	-	-	-	S	D	T	Q	H	S	R	V	L	S	L	Y	N	T	I	N	P	-	-	E	A	S	A	S	P		C		C		
TGF-β3:	P	Y	L	R	S	-	-	-	-	-	-	A	D	T	T	H	S	T	V	L	G	L	Y	N	T	L	N	P	-	-	E	A	S	A	S	P		C		C		
TGF-β4:	P	Y	I	W	S	-	-	-	-	-	-	A	D	T	Q	Y	T	K	V	L	A	L	Y	N	Q	H	N	P	-	-	G	A	S	A	A	P		C		C		
TGF-β5:	P	Y	I	W	S	-	-	-	-	-	-	M	D	T	Q	Y	S	K	V	L	S	L	Y	N	Q	N	N	P	-	-	G	A	S	I	S	P		C		C		
PATTERN:	P	Y	c	W	S	-	-	-	-	-	-	X	D	T	Q	e	S	n	V	L	j	L	Y	N	r	X	N	P	-	-	X	A	S	A	j	P		C		C		
Vg/dpp SUBGROUP-----+-----+-----+																																										
dpp:	P	F	P	L	A	D	H	F	-	-	-	N	S	T	N	H	A	V	V	Q	T	L	V	N	N	M	N	P	-	-	G	K	V	P	K	A		C		C		
Vg-1:	P	Y	P	L	T	E	I	L	-	-	-	N	G	S	N	H	A	I	L	Q	T	L	V	H	S	I	E	P	-	-	E	D	I	P	L	P		C		C		
Vgr-1:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	N	P	-	-	E	Y	V	P	K	P		C		C		
60A:	N	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	L	E	P	-	-	K	K	V	P	K	P		C		C		
BMP-2A:	P	F	P	L	A	D	H	L	-	-	-	N	S	T	N	H	A	I	V	Q	T	L	V	N	S	V	N	-	-	-	S	K	I	P	K	A		C		C		
DORSALIN:	F	F	P	L	T	D	N	V	-	-	-	T	P	T	K	H	A	I	V	Q	T	L	V	H	L	Q	N	P	-	-	K	K	A	S	K	A		C		C		
BMP-2B/BMP-4:	P	F	P	L	A	D	H	L	-	-	-	N	S	T	N	H	A	I	V	Q	T	L	V	N	S	V	N	-	-	-	S	S	I	P	K	A		C		C		
BMP-3:	Q	F	P	M	P	K	S	L	-	-	-	K	P	S	N	H	A	T	I	Q	S	L	V	R	A	V	G	V	V	-	P	G	I	P	E	P		C		C		
BMP-5:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	F	P	-	-	D	H	V	P	K	P		C		C		
BMP-6:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	N	P	-	-	E	Y	V	P	K	P		C		C		
OP-1/BMP-7:	A	F	P	L	N	S	Y	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	F	I	N	P	-	-	E	T	V	P	K	P		C		C		
OP-2:	S	F	P	L	D	S	C	M	-	-	-	N	A	T	N	H	A	I	L	Q	S	L	V	H	L	M	K	P	-	-	N	A	V	P	K	A		C		C		
OP-3:	I	Y	P	L	N	S	C	M	-	-	-	N	S	T	N	H	A	T	M	Q	A	L	V	H	L	M	K	P	-	-	D	I	I	P	K	V		C		C		
PATTERN:	X	F	P	L	X	X	X	b	-	-	-	N	j	T	N	H	A	I	a	Q	T	L	V	r	X	c	r		z	z	-	r	X	a	P	K	j		C		C	
GDF SUBGROUP-----+-----+-----+																																										
GDF-1:	A	L	P	E	T	L	R	G	P	G	G	P	P	A	L	N	H	A	V	L	R	A	L	M	H	A	A	A	P	T	-	P	G	A	G	S	P		C		C	
GDF-3:	P	F	S	M	T	T	Y	L	-	-	-	N	S	S	N	Y	A	F	M	Q	A	L	M	H	M	A	D	-	-	-	P	K	V	P	K	A		V		C		
GDF-9:	P	R	A	V	R	H	R	Y	-	-	-	G	S	P	V	H	T	M	V	Q	N	I	I	Y	E	K	L	D	-	-	P	S	V	P	R	P		S		C		
PATTERN:	j	X	j	X	r	X	X	X	z	z	z	X	j	X	X	e	j	f	c	p	X	c	c	e	X	X	X		z	z	-	P	X	X	j	r	j		X		C	
INHIBIN SUBGROUP-----+-----+-----+																																										
INHIBIN α:	G	L	H	I	P	P	N	L	S	L	-	-	P	V	P	G	A	P	T	P	A	Q	P	Y	S	L	-	-	-	L	P	G	A	Q	P		C		C			
INHIBIN βA:	P	S	H	I	A	G	T	S	G	S	-	-	S	L	S	F	H	S	T	V	I	N	H	Y	R	M	R	G	H	S	P	F	A	N	L	K	S		C		C	
INHIBIN βB:	P	A	Y	L	A	G	V	P	G	S	-	-	A	S	S	F	H	T	A	V	V	N	Q	Y	R	M	R	G	L	N	-	P	G	T	V	N	S		C		C	
PATTERN:	j	X	e	c	j	j	X	X	j	X	-	-	j	X	j	X	X	j	j	X	X	X	r	X	X	X	X	z		z	z	z	X	j	X	X	r	j		C		C
-----+-----+-----																																										

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TGF-β SUBGROUP																																										
TGF-β1:	V	-	-	P	Q	A	L	E	P	L	P	I	V	Y	Y	V	G	-	-	R	K	P		K	V	E	Q	L	S	N	M	I	V	R	S		C	K	C		S	
TGF-β2:	V	-	-	S	Q	D	L	E	P	L	T	I	L	Y	Y	I	G	-	-	K	T	P		K	I	E	Q	L	S	N	M	I	V	K	S		C	K	C		S	
TGF-β3:	V	-	-	P	Q	D	L	E	P	L	T	I	L	Y	Y	V	G	-	-	R	T	P		K	V	E	Q	L	S	N	M	V	V	K	S		C	K	C		S	
TGF-β4:	V	-	-	P	Q	T	L	D	P	L	P	I	I	Y	Y	V	G	-	-	R	N	V		R	V	E	Q	L	S	N	M	V	V	R	A		C	K	C		S	
TGF-β5:	V	-	-	P	D	V	L	E	P	L	P	I	I	Y	Y	V	G	-	-	R	T	A		K	V	E	Q	L	S	N	M	V	V	R	S		C	N	C		S	
PATTERN:	V	-	-	P	Q	X	L	E	P	L	j	I	c	Y	Y	V	G	-	-	R	r	j		K	V	E	Q	L	S	N	M	a	V	n	S		C	K	C		S	
Vg/dpp SUBGROUP																																										
dpp:	V	-	-	P	T	Q	L	D	S	V	A	M	L	Y		L	N	D	Q	-	S	T	V		V	L	K	N	Y	Q	E	M	T	V	V	G		C	G	C		R
Vg-1:	V	-	-	P	T	K	M	S	P	I	S	M	L	F		Y	D	N	N	-	D	N	V		V	L	R	H	Y	E	N	M	A	V	D	E		C	G	C		R
Vgr-1:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y		F	D	D	N	-	S	N	V		I	L	K	K	Y	R	N	M	V	V	R	A		C	G	C		H
60A:	A	-	-	P	T	R	L	G	A	L	P	V	L	Y		H	L	N	D	-	E	N	V		N	L	K	K	Y	R	N	M	I	V	K	S		C	G	C		H
BMP-2A:	V	-	-	P	T	E	L	S	A	I	S	M	L	Y		L	D	E	N	-	E	K	V		V	L	K	N	Y	Q	D	M	V	V	E	G		C	G	C		R
DORSALIN:	V	-	-	P	T	K	L	D	A	I	S	I	L	Y		K	D	D	A	G	V	P	T		L	I	Y	N	Y	E	G	M	K	V	A	E		C	G	C		R
BMP-2B/BMP-4:	V	-	-	P	T	E	L	S	A	I	S	M	L	Y		L	D	E	Y	-	D	K	V		V	L	K	N	Y	Q	E	M	V	V	E	G		C	G	C		R
BMP-3:	V	-	-	P	E	K	M	S	S	L	S	I	L	F		F	D	E	N	-	K	N	V		V	L	K	V	Y	P	N	M	T	V	E	S		C	A	C		R
BMP-5:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y		F	D	D	S	-	S	N	V		I	L	K	K	Y	R	N	M	V	V	R	S		C	G	C		H
BMP-6:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y		F	D	D	N	-	S	N	V		I	L	K	K	Y	R	N	M	V	V	R	A		C	G	C		H
OP-1/BMP-7:	A	-	-	P	T	Q	L	N	A	I	S	V	L	Y		F	D	D	S	-	S	N	V		I	L	K	K	Y	R	N	M	V	V	R	A		C	G	C		H
OP-2:	A	-	-	P	T	K	L	S	A	T	S	V	L	Y		Y	D	S	S	-	N	N	V		I	L	R	K	H	R	N	M	V	V	K	A		C	G	C		H
OP-3:	V	-	-	P	T	E	L	S	A	I	S	L	L	Y		Y	D	R	N	-	N	N	V		I	L	R	R	E	R	N	M	V	V	Q	A		C	G	C		H
PATTERN:	X	-	-	P	T	_p	L	r	A	a	S	c	L	Y		f	D	m	r	z	r	r	V		a	L	n	r	Y	p	I	M	X	V	p	j		C	G	C		r
GDF SUBGROUP																																										
GDF-1:	V	-	-	P	E	R	L	S	P	I	S	V	L	F		F	D	N	S	-	D	N	V		V	L	R	H	Y	E	D	M	V	V	D	E		C	G	C		R
GDF-3:	V	-	-	P	T	K	L	S	P	I	S	M	L	Y		Q	D	S	D	-	K	N	V		I	L	R	H	Y	E	D	M	V	V	D	E		C	G	C		G
GDF-9:	V	-	-	P	G	K	Y	S	P	L	S	V	L	T		I	E	P	D	-	G	S	I		A	Y	K	E	Y	E	D	M	I	A	T	R		C	T	C		R
PATTERN:	V	-	-	P	X	n	f	S	P	c	S	c	L	X		X	k	X	r	-	X	r	a		X	f	n	r	Y	E	D	M	a	X	r	p		C	j	C		X
INHIBIN SUBGROUP																																										
INHIBIN α:	A	A	L	P	G	T	M	R	P	L	H	V	R	T		T	S	D	G	G	Y	S	F		K	Y	E	T	V	P	N	L	L	T	Q	H		C	A	C		I
INHIBIN βA:	V	-	-	P	T	K	L	R	P	M	S	M	L	Y		Y	D	D	G	-	Q	N	I		I	K	K	D	I	Q	N	M	I	V	E	E		C	G	C		S
INHIBIN βB:	I	-	-	P	T	K	L	S	T	M	S	M	L	Y		F	D	D	E	-	Y	N	I		V	K	R	D	V	P	N	M	I	V	E	E		C	G	C		A
PATTERN:	X	z	z	P	j	r	b	r	j	b	r	c	X	X		X	r	D	X	z	X	r	f		X	X	p	r	a	X	N	b	c	X	o	r		C	h	C		X

FIG. 6C

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TGF- β SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |C|C V R P L Y I D|F R n D L|G W K - W I H E P K|G Y X A N F|C X G j C|
 Vg/dpp SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |C|n n r r L Y V r|F r - D c|G W r D W I I A P p|G Y X A d Y|C r G k C|
 GDF SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |C|r X r r f X c r|F r - r c|X W r r W a a A P r|X d X j r d|C r G r C|
 INHIBIN SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |C|X n X X f X a r|F p - X c|G W m r W I a X P j|j d X X r Y|C r G X C|
 +-----+-----+-----+-----+-----+
 1 10 20 30
 |K| BETA | HELIX | LOOP | BETA | RING
 |K| FINGER 1 |KNOT_A|

TGF- β SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |P Y c W S - - - - - X D T|Q e S n V L j L Y N r X N|P - - X A S A j P|C|C|
 Vg/dpp SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |X F P L X X X b - - - - N j T|N H A I a Q T L V r X c r|z z - r X a P K j|C|C|
 GDF SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |j X j X r X X X z z z z X j X|X e j f c p X c c e X X X|z z - P X X j r j|X|C|
 INHIBIN SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |j X e c j j X X j X - - j X j|X X j j X X X r X X X X z|z z z X j X X r j|C|C|
 +-----+-----+-----+-----+-----+
 40 50 60 70
 | HELIX |
 | HEEL | I |

TGF- β SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |V - - P Q X L E P L j I c Y|Y V G - - R r j|K V E Q L S N M a V n S|C K C|S|
 Vg/dpp SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |X - - P T p L r A a S c L Y|f D m r z r r V|a L n r Y p I M X V p j|C G C|r|
 GDF SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |V - - P X n f S P c S c L X|X k X r - X r a|X f n r Y E D M a X r p|C j C|X|
 INHIBIN SUBGROUP +-----+-----+-----+-----+-----+
 PATTERN: |X z z P j r b r j b r c X X|X r D X z X r f|X X p r a X N b c X o r|C h C|X|
 +-----+-----+-----+-----+-----+
 80 90 100 110
 | BETA | LOOP | BETA | RING
 | FINGER 2 |KNOT2C|

FIG. 7

